

Risk assessment of meat and milk from cloned animals

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Research on, and commercialization of, cloned cattle has been conducted for more than 20 years. Early techniques relied on the physical splitting of embryos or using embryo cells for nuclear transfer to generate cloned animals. Milk and meat from these animals entered into the human food market with no evidence of problems. With the advent of nuclear transfer, which enables the direct transference and preservation of high-value meat- and milk-producing genotypes to offspring, concerns have been raised about whether the products from somatic cell nuclear transfer-produced animals are safe for human consumption. Studies on the biochemical properties of food products from cloned and noncloned animals have thus far not detected any differences. All data to date indicate no significant differences in the measured parameters between animals created by nuclear transfer and normally bred animals. Public acceptance of cloned animal products depends upon forthcoming US Food and Drug Administration approval along with convincing safety data.

One role of government food regulatory agencies around the world is to insure that food supplies are safe. In animal agriculture, selective breeding and selection of natural high-merit animals has been used historically to improve the production and characteristics of the food supply. Several technological advances have been used to enhance this process, including artificial insemination and multiple ovulation/embryo transfer to amplify reproductive rates of superior males and females, respectively. Superimposed on these procedures is cryopreservation of sperm, oocytes and embryos. Newer methods in this continuum include *in vitro* fertilization, embryo cryopreservation and sexing embryos or sperm with subsequent artificial insemination (AI) and/or transfer of embryos to recipients for gestation to term, as recently reviewed by Bousquet and Blondin¹.

Embryo cloning by embryonic cell (blastomere) separation or embryo splitting also can be used to multiply animal embryos with superior

genetics; these technologies facilitate the 'horizontal' propagation of superior livestock (for meat, milk or both). Early sheep embryo cloning methods involved embryo cell separation of early fertilized preimplantation embryos to produce identical twins, triplets or quadruplets from single embryos². The same technology was later applied in cattle and further modified by surgical splitting of morula- and blastocyst-stage embryos for nonsurgical embryo transfer^{3,4}. These methods, indeed, produced genetically identical offspring, as they were derived from the same embryo, but with the practical limitation of a maximum of four copies in cattle and sheep^{4,5}.

A total of 1,536 cows and 783 bulls created by embryo splitting were registered by the Holstein Association USA, Brattleboro, VT, USA between 1982 and October 2002 (refs. 6,7). The association maintains blood typing and embryo transfer information on registered animals, which allowed accurate identification data for several studies on the composition of milk from embryo transfer and nuclear transfer clones^{6,7}.

The relatively new technology of nuclear transfer cloning has been around for only ~20 years in sheep and cattle, starting in the mid-to-late 1980s (ref. 6). These methods used cells from early embryos as the source of donor nuclei that were then transplanted into enucleated oocytes⁸ to create embryonic cell nuclear transfer (ECNT) clones. Some oocytes came from ovaries obtained from slaughterhouses. These oocytes then underwent *in vitro* maturation, whereas others were collected as *in vivo*-matured oocytes from within the oviducts of live adult cows.

Approximately 1,200–1,500 cows and bulls were produced by ECNT in North America in the 1980s and 1990s. These cloned dairy and beef cattle were produced for research or commerce, and most were used for human food consumption with no public knowledge or regulatory review. Offspring continue to be produced by embryo splitting and ECNT, and enter the human food chain with little public awareness and no regulatory review.

A significant breakthrough in animal cloning technology came when Wilmut *et al.*⁹ reported successful nuclear transfer using somatic cells derived from adult tissue (somatic cell nuclear transfer; SCNT) in mammals (sheep) in 1997. Technologically, SCNT and conventional ECNT are identical, with the exception that adult somatic cells rather than embryonic cells are used as nuclear donors. SCNT has potential applications in animal agriculture (e.g., both for commercial meat/dairy production and for generation of breedstocks with agronomically important genetic traits for use in developing nations with poor-quality animals), saving endangered species and ultimately in producing immune-matched human cells for therapies.

Some problems with animals produced by SCNT or ECNT have raised human safety and animal health-related concerns. From the animal

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Table 1 FDA suggested analyses of milk and meat for comparison between clones and noncloned livestock (adapted from ref. 18)

Product properties	Milk	Meat
Vitamins and minerals	Vitamins A, C, B1, B2, B12, niacin, pantothenic acid, calcium, iron, phosphorus	Vitamins A, C, B6, B12, niacin, calcium, iron, phosphorus, zinc
Fatty acids	Saturated and unsaturated	Saturated, unsaturated and cholesterol
Proteins	Protein characterization (casein, whey breakdown) and amino acids	Protein characterization and amino acids

welfare standpoint, these include a poor success rate for embryo development to term, 'large offspring syndrome' (where cloned lambs and calves are often large at birth^{1,10–13}), together with other symptoms, such as placental abnormalities, edema, large umbilicus or perinatal deaths. Many of these animal welfare issues have already been reviewed^{12,14,15}. Even so, concerns remain about the safety of cloned animal products for human consumption.

A 2002 report by the US National Academy of Sciences noted that the available science-based data were insufficient to address public concerns that such animal products are safe for human consumption, and further noted that, in the absence of analytical tests, there was no way to address public concerns about these food products¹⁶. In a preliminary report in 2003, the US Food and Drug Administration (FDA) indicated that there were no concerns about food safety for products from healthy adult bovine clones, swine clones, goat clones and the offspring of animal clones (<http://www.fda.gov/cvm/Documents/CLRAES.pdf>). A recently published paper signified the FDA's intent to allow meat and milk from cloned bovines, swine and goats and their offspring to enter the market without specific regulations or requirements for identification as clones or cloned offspring¹⁷. In this paper, Rudenko and Matheson¹⁷, from the Center for Veterinary Medicine at the FDA, state, "...food from these animals will not pose any additional risks compared with, and is as safe to eat as food from, conventionally bred animals"¹⁷. However, the document also noted that there was insufficient information to show that products of sheep clones will be safe.

In 2004, Rudenko and colleagues¹⁸ also discussed possible nutritional risks from meat or milk from cloned animals and ways to measure the composition of these products that could indicate their similarity to those that currently enter the food supply (Table 1). They pointed out that the risk would be from "inappropriate reprogramming" of the donor cell nucleus that may result in subtle changes in the composition of meat or milk. These changes could possibly decrease or eliminate certain vitamins or minerals in the muscle or milk, changing their nutritional profile, although not rendering the products unsafe. They suggested that certain components of milk and meat could be measured and used to show that there is no material difference between cloned and noncloned animal food products (Table 1).

We and others have been collecting data to determine if meat and milk from cloned cattle are indistinguishable from normally reproduced cattle using available standard tests. If these products are indistinguishable, it would imply that they are likely to be as safe as our standard food supply. We present here supporting evidence from the literature and from the most recent data obtained by our groups (Box 1, Fig. 1) that the composition of meat and milk from clones is biologically, biochemically and structurally the same as such products from normally reproduced animals.

The health of animals produced by embryo splitting or ECNT

Procedures for embryo cloning are based on modification of the work of Willadsen^{19–21} and involve using embryonic cells, taken primarily from morulae, as nuclear donors. The resulting pregnancies and calves exhibit a spectrum of problems subsequently seen with calves cloned from somatic cells, including low pregnancy rates, high levels of embryonic and fetal wastage during pregnancy and large offspring syndrome^{14,20,22,23}. These problems appear to have occurred at a similar or even a higher rate than those now observed in bovine pregnancies produced with somatic cell clones^{22,23}. Thus, the issues in reprogramming embryonic cell nuclei appear to be broadly similar to those in reprogramming somatic cell nuclei.

ECNT-cloned animal products

Nearly all the animals cloned from embryo cells eventually were slaughtered for meat for human consumption; many were dairy breeds and were milked during their productive years⁶ before being culled for meat. More than 300,000 kg of meat, and more than two million liters of milk from cloned cattle have probably entered the food supply (see **Supplementary Data** online). These cloned animal products were mixed with noncloned animal products, so minor problems would have gone undetected. Furthermore, no data were collected on health-related issues after consumption of such meat or milk products, so any statement on effects is conjecture.

A few studies have been published on the properties of the meat and milk from embryo-split or ECNT-generated clones (Table 2). Norman and colleagues^{6,7} analyzed data on file with the Holstein Association USA on properties of milk from 13 ECNT-cloned Holstein cows, together with those from 23 full sisters of these cloned cows as non-cloned controls, and compared them with Holstein Association data on 608 embryo-split clones and 1,034 full sisters of the embryo-split clones. They also compared means of total milk production, and milk fat and protein content for all embryo-split Holsteins versus noncloned Holsteins, and ECNT-cloned Holsteins versus noncloned Holsteins^{6,7}. They found no significant differences between ECNT and some SCNT clones and nonclones with respect to percent fat, percent protein, somatic cell score (a measure of mastitis), percent lactose, percent total solids, pH or in the total amounts of these components produced during lactation. The only significant difference was the amount of palmitic acid in the milk, and that likely was due to differences in feed composition at the different locations in which these animals were raised. They also found no differences between embryo-split clones and their full sisters.

Data on carcass characteristics of cattle cloned from embryonic nuclei were obtained by Diles *et al.*²⁴. They studied numerous characteristics of fat, muscle and bone of carcasses of eight steers, two each from four clonal sets. Their main objective was to study within- and between-clone variation. The data they obtained were within the normal range of carcass characteristics for cattle, including such traits as amounts of intramuscular, kidney, pelvic and heart fat as well as amount of muscle.

Most of these ECNT cloned cattle were valuable breeding animals that almost certainly produced thousands of progeny. Although we are not aware of any systematic study of progeny from the ECNT embryo-cloned animals, there have been no reports of reproductive problems with the cloned animals or their progeny.

In summary, cattle derived from embryo cloning—whether via split embryos or ECNT—have been accepted in animal agriculture as high genetic-merit breeding stock and as sources of food products for over 20 years. There are no reports on health—good or bad—of humans who have consumed meat or milk from those cloned animals.

SCNT-cloned animal health

In adult SCNT cloning, during the first few hours after the somatic cell fuses with the egg, until at least early embryonic development, the genome of the future clone is subject to massive epigenetic reprogramming. Using microarrays, we found that by the blastocyst stage, essentially all the analyzed genes in differentiated donor somatic cells were significantly reprogrammed to an undifferentiated totipotent embryonic cell stage, similar to what is found in naturally fertilized embryos²⁵.

Despite several studies in the literature describing anomalies during fetal development or in neonates^{10,11,26–29}, as in the embryo cell–cloned animals described above, most surviving SCNT-generated animals do well^{30–32}. SCNT cloned cattle calves beyond six months of age have presented no health issues of concern^{12,15,33}, but problems do exist in some bovine clones before reaching the six-month milestone. Several groups, including ours, have reported placental abnormalities, fatty livers and cardiovascular failure, as well as immaturity of the lungs²⁶. There is one report on the death of a newborn calf due to immune deficiencies²⁷.

Analyses of the long-term consequences for bovine SCNT clones are in progress. However, to date, all physiological parameters measured in clones beyond one year of age are similar to their naturally reproduced cohorts^{10,28,34,35}. We have used cloned versus age- and breed-matched naturally reproduced control cattle to compare growth rates³⁶, growth-related hormone profiles³⁷, age at puberty and related endocrinology³⁸, reproductive capacity and follicular profiles³⁸, epigenetic reprogramming (X-linked and imprinted genes)^{29,39,40}, telomere lengths⁴¹, behavior⁴² and growth profiles to adulthood⁴². All surviving animal clones appear

to be normal compared with naturally reproduced animals. Additionally, fertility of the offspring of the cloned animals of the cloned and re-cloned generations does not seem to be affected. When the offspring of a female and male clone was compared with that of their founder animals, no differences were observed in their growth performance, clinical parameters, serum biochemistry and telomere length in their chromosomes^{43,44}; similar results were reported in mice and pigs^{12,45,46}.

Our current state of knowledge indicates that, as bovine clones grow and mature, their health problems are not different from normally produced cattle. This is in agreement with findings reported by others for cloned cattle, goats and pigs^{47–49}. Rudenko and Matheson¹⁷ point out that there is little information available about the health of sheep clones; in addition, Rhind *et al.*⁵⁰ have reported pathologies that led to the deaths of SCNT-cloned sheep younger than six months of age. Although Wells and colleagues^{51,52} report increased annual death rates due to euthanasia of cloned cattle with musculoskeletal abnormalities, Rudenko and Matheson¹⁷ note that cloned cattle are otherwise healthy.

SCNT-derived clone products

Several researchers, including us, have compared the biological and biochemical qualities of meat and milk from cloned animals with meat and milk from noncloned animals^{53–55} (Table 2). Walsh and colleagues⁵⁵ compare total fat, fatty acid, nitrogen, solids, lactose, pH, acid degree value, protein profile and the elements Na, Ca, S, K, Zn, Fe, Sr and P in cloned and noncloned Brown Swiss, cloned and noncloned Holstein, and cloned and noncloned Holstein/Jersey cows. They found that all

Box 1 New findings on the blood chemistry and meat composition of SCNT cloned cattle

The commercial cloning company Cyagra (Elizabethtown, PA, USA) has maintained several SCNT-cloned animals at different commercial farms under different nutrition and management conditions. Although data obtained from these animals are not a standard research comparison between cloned and noncloned cattle maintained under the same conditions, they do provide a rough estimate of typical management practice for US cattle and what commonly is seen in US meat supplies from cattle slaughterhouses. Because we are in a unique position, having produced cloned cattle for research and commercial purposes, we have been able to make direct comparisons between cloned and noncloned cattle, with the ultimate goal of determining if these animals themselves, along with their milk and meat, are physiologically and biochemically comparable to noncloned animals.

To accomplish this, we had cattle produced by SCNT and noncloned control cattle slaughtered and sent for carcass analysis (11 cloned cattle versus 11 controls; Fig. 1 and Supplementary Table 1). All animals were older than 12 months of age, as commonly collected by US cattle slaughterhouses. Before slaughter, blood samples were collected and a complete blood cell count (hemogram) and routine blood chemistry analyses were conducted, including measurements of insulin-like growth factor-1 (IGF-1), which has previously been measured³⁷ and cholesterol concentrations. These are standard parameters measured by the commercial meat industry in a US Department of Agriculture–certified laboratory. At slaughter, each carcass was inspected for anatomical or other apparent defects. Three beef cuts (tenderloin, bottom round roast and chuck arm roast) were selected from each animal and frozen for analysis. Blood samples (separated serum, whole blood-EDTA and blood smears) were analyzed by the diagnostic laboratory at the Cornell University College of Veterinary

Medicine (Ithaca, NY, USA) using their standard animal health analysis protocols (<http://www.diaglab.vet.cornell.edu/labsect/neas.asp>). Meat composition analysis was performed by Eurofins, Inc. (Memphis, TN, USA).

For both SCNT cloned and noncloned cattle, we did not observe any anatomical or other apparent defects in any carcass inspected after slaughter. Results from the blood chemistry and routine hematology analyses are shown in Figure 1a. For meat composition, proximate analyses (water, fat, protein and carbohydrate content), amino acids, fatty acids and vitamins/minerals/cholesterol, average values are presented in Figure 1b. The standard deviations are similar for cloned and noncloned cattle for essentially all traits, which means that the variation is similar for characteristics of clones and controls (Fig. 1a,b).

This information can be used for statistical power tests concerning differences in means—in other words, how large a difference would be detectable (if there truly were such a difference between clones and controls) with the numbers of animals in these data sets. With assumptions of $\alpha = P < 0.05$, a 1-tail *t*-test, and $\beta = 0.2$ (80% power), the magnitudes of the differences detectable would be very close to the magnitudes of the standard deviations in Figure 1a,b. From these considerations, we can conclude that outlier animals with problems in product composition are no more likely to occur in cloned animals than in control animals. Although there was minor variability within and between naturally reproduced or cloned animals, no statistical difference was observed between cloned and control animals for any parameter analyzed. Furthermore, had there been a substantive difference between the means of clones and controls, there would be a high probability that such differences would have been detected statistically.

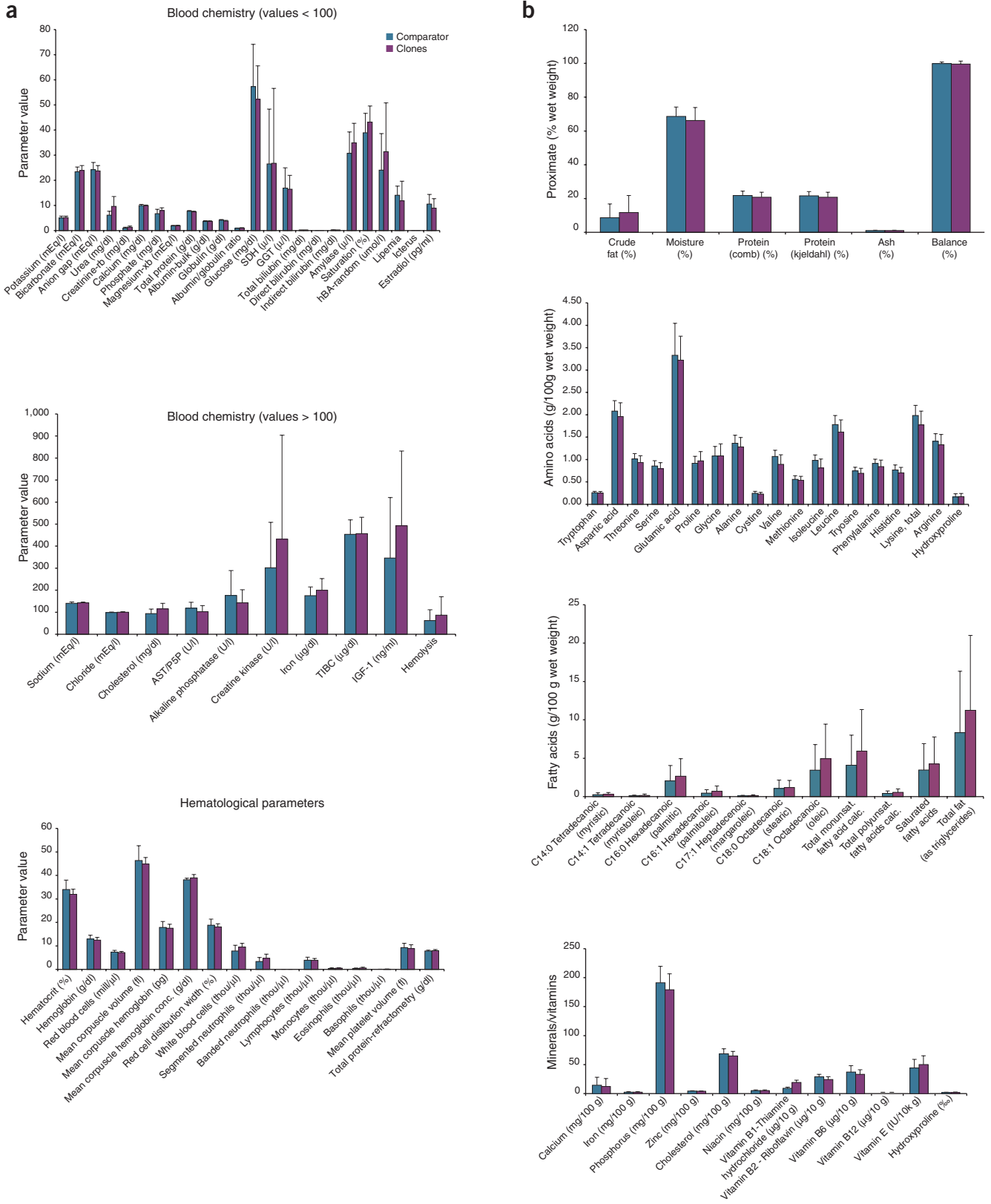


Figure 1 Cloned cattle versus controls: blood and meat composition. **(a)** Blood chemistry analysis and hematological analysis of bovine somatic cell clones. Comparison parameters from top to bottom: blood chemistry or hematological parameters. **(b)** Meat composition analysis based on wet weight. Error bars, s.d.

product composition parameters were within the normal food industry range, and that any product differences between cloned and noncloned animals could be attributed to animal breed, feed, season and differences in lactational stage. These differences are minor and reflect natural differences among conventionally bred animals. Major reprogramming problems would cause the lack of one or more components of milk or meat. Takahashi and Ito⁵⁶, as well as Tomé *et al.*⁵⁴, carried out preliminary studies feeding rats with milk or meat from cloned or control cows. They, too, found no major differences in the nutritional value or biological and biochemical properties between the milk and/or meat from cloned animals versus noncloned animals.

Recently, we measured more than 100 commonly used food industry parameters, including amino acid composition, fatty acid composition, proportion of meat and fat, organ weight and organ histopathology⁵³. All parameters measured for the milk from four cloned cows and meat from the two cloned bulls (that is, the world's first clones of a top-breeding beef bull for meat³⁰) fit the food industry normal ranges and did not differ significantly from meat and milk from naturally reproduced control cattle (two 50% genetic-matched controls and 20 breed-matched controls). Cloned high-merit beef bulls had significantly higher muscular fatty acids merit scores (a commonly used Japanese beef industry

standard) (http://www.geneticsolutions.com.au/files/GeneSTAR/pdf/GeneNOTE_6.pdf#search=japan%20meat%20grading%20association%20and%20marbling%20score) than those from the age-/breed-matched controls, undoubtedly due to the genomic inheritance of the donor bull's superior genetics for these parameters⁵³. Likewise, Takahashi and Ito⁵⁶ studied biochemical characteristics of meat samples from cloned and noncloned cattle and also found that there were no identifiable differences in amino acid and fatty acid composition, digestibility or allergenicity. Yonai *et al.*⁵⁷ compared milk yields and presence of fats, proteins and nonfat solids between cloned Jersey cows and their somatic cell donor, and cloned Holstein cows and their somatic cell donor. They found differences in total milk yields, but not in the percentages of fat, protein and solids-not-fat between clones and donors. Within each group, however, the comparison was only to a single noncloned donor; furthermore, environmental factors may have resulted in these differences, so the data are inconclusive. The protein and fat percentages in the milk from their cloned cows are within the normal ranges for milk composition, as published by the Florida Cooperative Extension Service⁵⁸. Aoki *et al.*⁵⁹ report that composition of milk from two cloned cows resembled that from eight cows produced via AI in percentages of milk fat, milk protein, milk lactose, nonfat solids and total solids.

Table 2 Chemical, biological, physiological and biochemical characteristics of blood, muscle and milk of cloned cattle versus noncloned animals^a

Method	Species	n (clones)	Tissue sampled	Parameters tested	Clone versus normal	Ref.
Composition analysis						
Embryo splitting	Cattle	608	Milk	% total fat and protein; SCS	Similar	6
ECNT	Cattle	13	Milk	% total fat and protein; SCS	Similar	6
ECNT	Cattle	1	Carcass	% total water, protein, lipids, carbohydrates, ash and cholesterol; profiles of amino acids; profiles of fatty acid	Similar	56
ECNT	Cattle	8	Carcass	% total fat, muscle and bone; intramuscular, kidney, pelvic and heart fat, and amount of muscle	Within normal industry range	24
SCNT	Cattle	12	Milk	% total solids, fat, protein and lactose; PH; acid degree value; somatic cell count; protein profiles, fatty acid profiles, mineral profiles	Similar	55
SCNT	Cattle	4	Milk	% total protein, fat, solids, lactose, milk urea nitrogen and SCS; protein profiles	Similar	53
SCNT	Cattle	10	Milk	Quantity; % total fat, protein and solids-not-fat	Differences in quantity; otherwise similar	56
SCNT	Cattle	2	Milk	Quantity; % milk fat, protein, lactose, solids-not-fat, total solids	Similar	59
SCNT	Cattle	2	Carcass	Organ or body part weights; % total meat and fat; % moisture, crude protein and crude fat in six muscles; profiles of fatty acids; amino acids composition; histopathology of all organs	12 out of over 100 parameters different, but all within normal industry range	53
SCNT	Cattle	1	Carcass	% total water, protein, lipids, carbohydrates, ash and cholesterol; profiles of amino acids; profiles of fatty acid	Similar	56
SCNT	Cattle	11	Blood and carcass	Blood chemistry, hematology parameters, composition of fatty acids, composition of amino acids, vitamins and proximate	Similar	Present report
SCNT	Cattle	18	Blood	Blood chemistry parameters	6 out of 592 parameters different	18
Feeding studies						
ECNT	Cattle	1		Meat digestibility, allergenicity and mutagenicity (after feeding to mice and rats)	Similar	56
SCNT	Cattle	1		Meat digestibility, allergenicity and mutagenicity (after feeding to mice and rats)	Similar	56
SCNT	Cattle	Unknown		Body composition and fasting glycemia of rats fed by milk from cloned cows	Similar	54

^aResults from literature or from studies in which products of cloned cattle were fed to mice. SCS, somatic cell score; ECNT, embryonic cell nuclear transfer; SCNT, somatic cell nuclear transfer; SNF, solids not fat.

Overall, to date, eight published studies, not including the present report (Box 1) and the data cited in the FDA's Rudenko and Matheson report¹⁷, as listed in Table 2, document that cloned cattle show no biological or biochemical differences in their meat or milk in comparison to noncloned cattle using conventional tests.

In summary, these studies showed that values for all milk components do not differ significantly between cloned cattle and conventionally bred noncloned cattle and that all parameters commonly used for detailed meat composition analyses are indistinguishable among all cloned and noncloned animals. This present study (Box 1 and Fig. 1a) shows that values for blood components commonly used for veterinary medical practice to diagnose disease do not differ between cloned and noncloned cows. Additionally, a detailed carcass analysis of parameters used in the meat industry to biochemically quantify meat composition did not reveal any significant differences either between cloned and noncloned cattle (Box 1 and Fig. 1b). Overall, published studies (summarized in Table 2), along with data from this present report (Box 1 and Fig. 1) document that cattle clones show no biological or biochemical differences in their meat or milk in comparison to noncloned cattle.

Conclusions

Despite its current high cost^{1,60}, SCNT cloning of farm animals is the only tool available that can guarantee survival of the highest-value animal lines that are genetically identical to the specific, well-known highest-value parents, such as top-breeding bulls. Selected breeding by AI using top genetic-merit males and females is commonly used for selecting potential top-breeding bulls based on their progeny's product tests (milk or meat for dairy or beef cattle, respectively). However, this commonly used breeding strategy routinely takes 7–8 years to prove the parents' potential; in the United States, only 0.1% of male dairy calves may be selected based on their family pedigrees as young breeding bull candidates. Among those young candidate breeding bulls that have genetically top-merit parents, only 5–10% of them are finally selected as truly valuable breeding bulls on the basis of their daughters' milk production quantity and quality.

To date, almost all studies on the biochemical composition of the meat and milk of cloned cattle have shown that these products cannot be differentiated physically, physiologically or biochemically from the meat and milk from noncloned cattle. Although animal cloning through embryo splitting and ECNT has been a standard, albeit not tremendously common, practice in cattle breeding for more than 20 years, there have been no reports of problems from these animals entering the food supply. It is also important to note that all currently used cloning methods for producing food animals differ from transgenesis in that the cloned animal's genome does not undergo genetic alteration at the level of sequence. All changes that occur to the cell's nuclear DNA result from epigenetic changes affected by the interaction of the adult cell nucleus with the oocyte cytoplasm.

Taken together, the numerous reports in the literature of the composition of milk and meat from animals produced by various means and our own new data presented in this article provide compelling evidence that the composition of milk and meat from cloned animals does not differ significantly from milk and meat from noncloned animals produced by the usual fertilization mechanisms (natural fertilization or AI).

Note: Supplementary information is available on the Nature Biotechnology website.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the Nature Biotechnology website for details).

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